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Physicochemical and Phytochemical Evaluation of Siddha formulation Saaranai chooranam

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ABSTRACT

Background: Siddha system of medicine depends largely on herbal for the treatment of diseases which was practiced at village levels and now becomes an important medicine in worldwide, According to the Akasthiyar -2000-Part– III textbook, Saaranai Chooranam is a herbal drug that is indicated for *Raththa kothippu* (Systemic Hypertension) Saaranai is the Tamil name for *Trianthema portulacastrum*, which belongs to the family Aizoaceae. **Aim & Objective**: This study primarily aims to evaluate the Physicochemical and Phytochemical evaluation of Saaranai Chooranam, Preliminary Phytochemical analysis such as High Performed Thin Layered Chromatography and Powder Microscopy of Saaranai Chooranam. **Methodology**: The Physicochemical analysis of Saaranai Chooranam is carried out using standard procedures. **Results**: Preliminary Phytochemical screening found, the presence of phytochemicals such as Proteins, Terpenoids, Alkaloids, Carbohydrates, and Tannins. High-performance thin-layer chromatography fingerprinting revealed the presence of many phytochemicals with different Rf values and densitometric scans of the plates showed numerous bands and peaks. The Powder Microscopy reveals the presence ofTracheidal fibre, Vessel with bordered pits, Calcium oxalate crystal, Stone cell, Group of sclereids with brownish content, Paracytic stomata and surrounding subsidiary cells, Rosette Calcium oxalate crystal, Sclereid with narrow lumen, Thick walled cells, Tracheidal fibre and Trichome. Physicochemical analysis revealed the values of total ash (17.86%), acid-insoluble ash (1.34%), water-soluble ash (13.62), sulphated ash (25.72%), pH (6.05 in 4% alcohol solution), volatile oil (0.5%), foaming index (111.11%) and swelling index (4ml). **Conclusion**: This study is an effort to explore the different Physico and Phytochemical compounds of Saaranai Chooranam effective in the management of Raththa kothippu (Systemic hypertension).

Keywords: Akasthiyar-2000; Physicochemical; High-performance thin layer Chromatography; *Raththa kothippu*.

1. Introduction

The Siddha system of medicine consists of many herbal, herbo-mineral and metallic preparations. Herbal preparations gained worldwide attention due to their long-term benefits in terms of overall wellness with lesser side effects. Systemic Hypertension is a Silent killer of society. It is a long-term serious medical condition in which the blood pressure in the arteries is persistently elevated and also known as High blood pressure.

According to *Yugi Vaidhya Chinthamani* - 800, *Siddha Maruthuvam (Sirappu*) and *Noi Naadal Noi Muthal naadal Thiradu Pitta* diseases are classified into 42 types, among them *Raththa kothippu* is one of the *pitta* diseases. The symptoms mentioned under *Raththa kothippu*in *Noi Naadal Noi Muthal Naadal Thiraddu* can be correlated with Systemic hypertension (SHT) in the modern aspect. According to the Akasthiyar-2000, Part – III textbook, Saaranai Chooranam is a herbal drug that is indicated for *Raththa kothippu* (Systemic hypertension) and Piththa diseases. Saaranai is the common name for *Trianthema portulacastrum* in Tamil Literature. Which belongs to the family Aizoaceae, commonly known as "Giant pigweed" grows in a wide variety of habitat types and distributed areas and cultivated land as a weed.

The present study was undertaken to analyze the Physicochemical and Phytochemical evaluation of Saaranai Chooranam and the work was carried out at Siddha Research Regional Institute, Thiruvananthapuram.

2. Materials and Methods

The root of Saaranai was collected from in and around Tirunelveli. It was identified and authenticated by the Medicinal botanists at GSMC, Palayamkottai. Method of preparation, Saaranai root is thoroughly washed in water

and soaked in cow's milk. After that it steamed in milk. Dried and grind into the fine powder sieved and add same quantity of Inthuppu. The drug will be labelled as Saaranai Chooranam.

2.1. Physicochemical analysis

The physicochemical analysis such as determination of loss on drying, total ash value, acid insoluble ash, water soluble ash, sulphated ash, pH value, volatile oil, alcohol soluble extractives, water soluble extractives were carried out by standard methods. The information collected from these tests are used for standardization.

2.2. Preliminary Phytochemical Analysis

Preliminary phytochemical screening was carried out to find out the presence of various phyto constituents using standard procedures.

2.2.1. High Performance Thin Layer Chromatography

High Performance Thin Layer Chromatography is a popular method for the quality control of herbal products and the analysis of herbal medicines. It is widely used for separation. qualitative and quantitative estimation of marker compounds present in herbal drugs. HPTLC fingerprint profile is suitable for standardization of components followed by determination of specific bio-active phyto constituents from plant materials.

Preparation of the alcoholic extract of the drug for HPTLC analysis

Five gram of the powdered sample is taken and reflux with200ml of alcohol using a soxhlet apparatus on a water bath for 30 minutes. Filter the extract and concentrate to 5ml then the sample extract obtained is used for further experimental studies.

Procedure

Developing solvent system: A number of solvent systems were tried and a system which gave the maximum resolution was selected as the solvent system for the extract. The optimum separations of constituents were achieved using the specified solvent system.

Sample application: The extracts were applied as different tracks of different concentrations of width 8 mm each on silica gel 60 F254 pre coated aluminium sheets through CAMAG micro litre syringe using Automatic TLC Sampler 4 (ATS4). Development of chromatogram. After sample application plate was introduced vertically in a CAMAG developing chamber (10 cm × 10 cm) pre-saturated with the mobile phase selected.

Documentation: The developed chromatogram was air dried to evaporate solvents from the plate and the plate was kept in CAMAG Vizualizer and the images were captured under UV light at 254 nm and 366 nm.

Densitometry: The plate was scanned at 254 nm and 366 nm using TLC Scanner 4 and the finger print profiles were documented. The Rf values and finger print data were recorded with win CATS software associated with the scanner. Post chromatographic derivatisation: The plate was derivatized using vanillin-sulphuric acid reagent, heated at 105^0C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and the chromate gram swere documented. The plate was scanned at 575 nm and the Rf values and finger print data were documented.

2.2.2. Powder Microscopy

About 0.5gm of the powdered sample was mounted in glycerin at room temperature for 2 h and observed under 10X and 40X objective of bright field microscope for powder characteristics. Photomicrographs of diagnostic characters were captured using attached camera.

3. Results and Discussion

3.1. Results of Physicochemical Analysis

Table 1. Physicochemical Analysis of Saaranai Chooranam

Sl. No.	Parameters	Result
1	LOD at 105^0C	7.68%
2	Total Ash	17.86%
3	Acid insoluble ash	1.34%
4	Water soluble ash	13.62%
5	Sulphated ash	25.72%
6	pH of water extract	6.05
7	Volatile oil	0.5%
8	Alcohol soluble extractives	11.93%
9	Water soluble extractives	18.02%
10	Swelling index	4.0
11	Foaming index	111.11

3.2. Results of Phytochemical Analysis

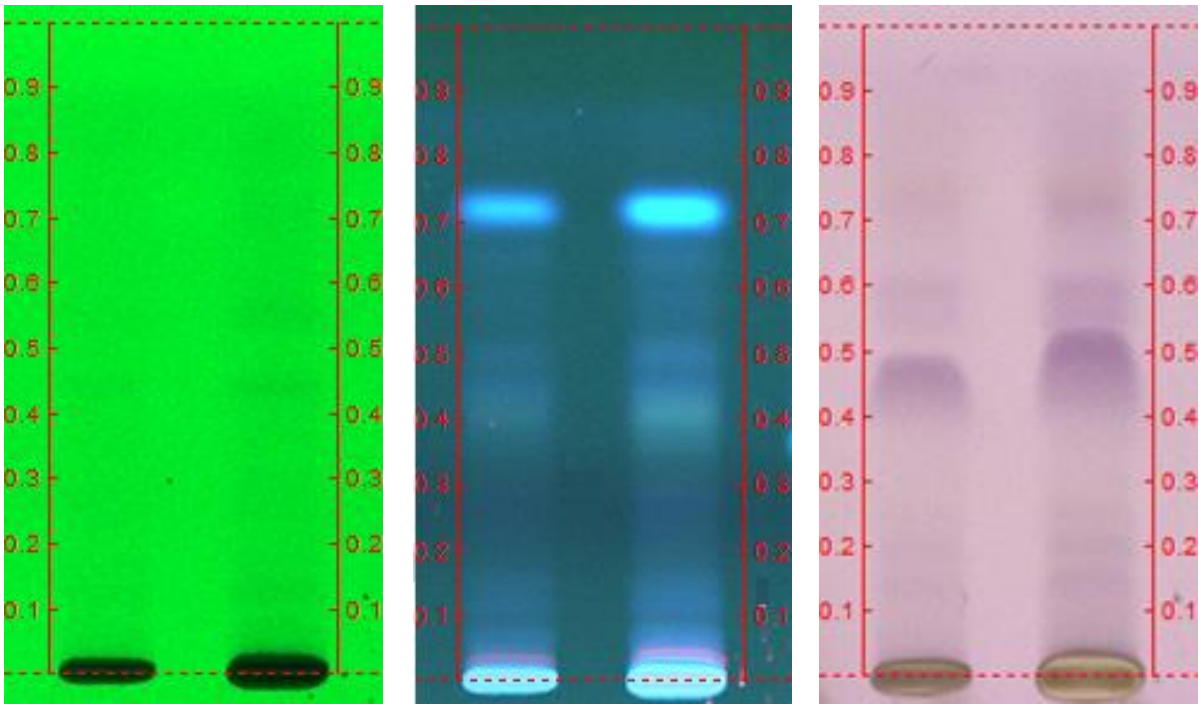
Table 2. Phytochemical analysis of Saaranai Chooranam

Tests	Results
Saponins	-
Tannins	+
Phenols	-
Terpenoids	+
Alkaloids	+
Flavanoids	-
Steroids	-

Glycosides	-
Carbohydrates	+
Quinones	-
Proteins	+

3.3. HPTLC Analysis

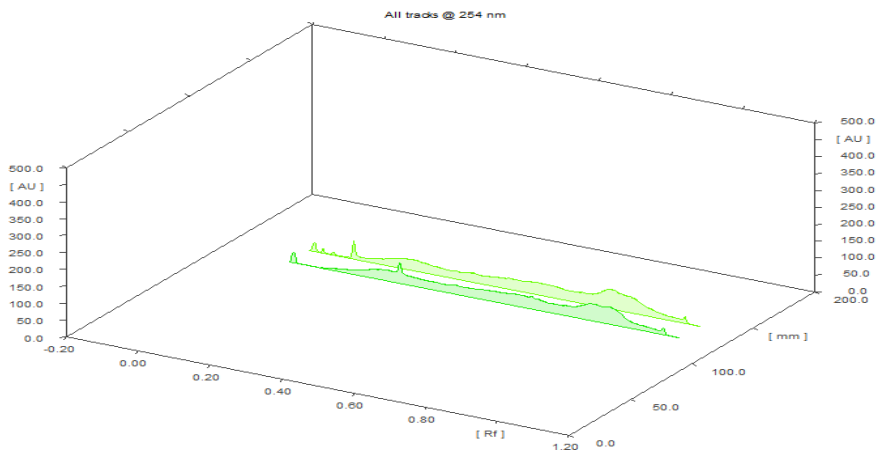
Solvent system: Toluene: Ethyl acetate (5:2), Track 1,2: Saaranai chooranam, Volume applied: 5, 10 µl each.

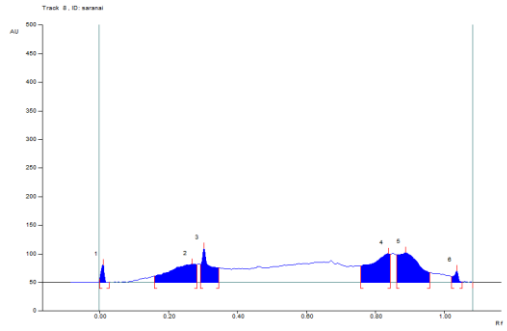


Under UV short Under UV long Under white light

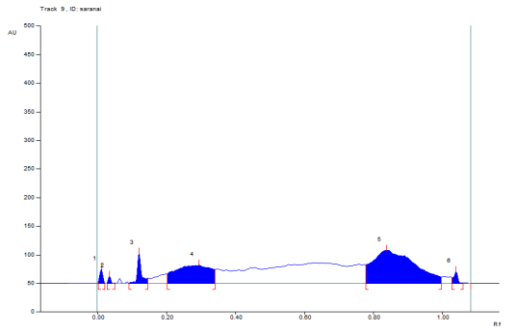
Figure 1. HPTLC profile of alcohol extract of Saaranai chooranam viewed in UV short; UV long; White light after derivatization using the Solvent system: Toluene: Ethyl acetate (5:2); Track 1, 2: Saranai chooranam; Volume applied: 5, 10 µl each.

254 nm





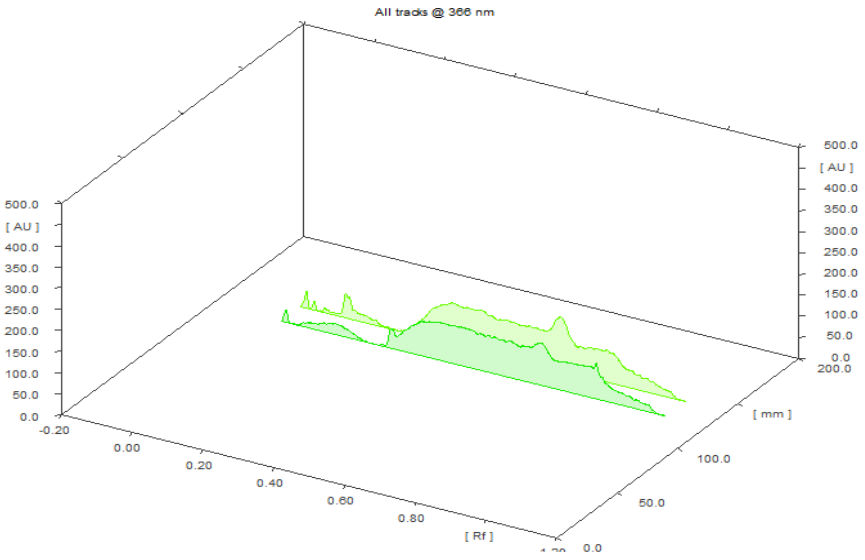
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	6.1 AU	0.01 Rf	31.2 AU	12.60 %	0.03 Rf	0.5 AU	217.2 AU	2.89 %
2	0.16 Rf	10.9 AU	0.27 Rf	32.1 AU	12.95 %	0.28 Rf	31.3 AU	1744.8 AU	23.22 %
3	0.29 Rf	31.5 AU	0.30 Rf	60.1 AU	24.26 %	0.35 Rf	25.5 AU	1089.8 AU	14.50 %
4	0.76 Rf	28.8 AU	0.84 Rf	51.0 AU	20.59 %	0.85 Rf	49.3 AU	2033.7 AU	27.06 %
5	0.86 Rf	48.3 AU	0.89 Rf	52.3 AU	21.10 %	0.96 Rf	17.1 AU	2231.6 AU	29.69 %
6	1.02 Rf	10.3 AU	1.04 Rf	21.0 AU	8.50 %	1.05 Rf	0.6 AU	198.6 AU	2.64 %

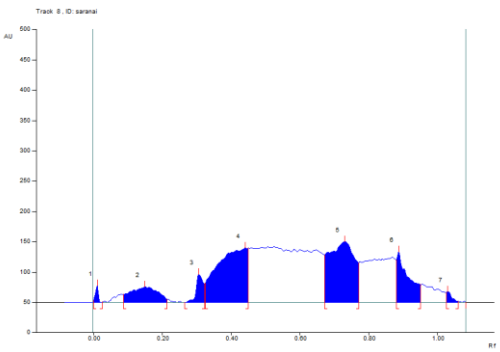


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	1.7 AU	0.01 Rf	24.9 AU	12.39 %	0.02 Rf	0.5 AU	172.1 AU	2.12 %
2	0.03 Rf	0.5 AU	0.04 Rf	12.9 AU	6.43 %	0.05 Rf	0.9 AU	61.8 AU	0.76 %
3	0.09 Rf	1.0 AU	0.12 Rf	52.3 AU	26.07 %	0.15 Rf	9.0 AU	440.5 AU	5.44 %
4	0.20 Rf	16.4 AU	0.29 Rf	31.7 AU	15.78 %	0.34 Rf	24.3 AU	2290.6 AU	28.27 %
5	0.78 Rf	33.1 AU	0.84 Rf	58.3 AU	29.08 %	1.00 Rf	11.6 AU	4962.1 AU	61.23 %
6	1.03 Rf	10.6 AU	1.04 Rf	20.6 AU	10.26 %	1.06 Rf	0.6 AU	176.3 AU	2.18 %

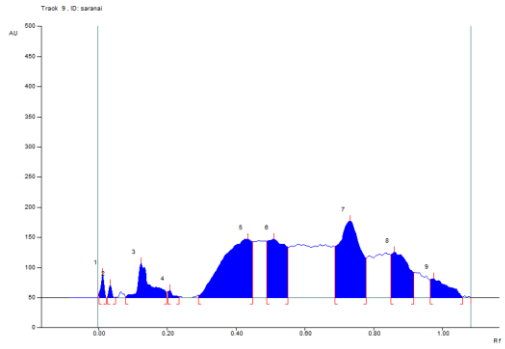
Figure 2. HPTLC fingerprint profile of 5 µl and 10 µl of alcohol extract of Saaranai chooranam at 254nm after derivatisation

366 nm





Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	3.6 AU	0.01 Rf	27.6 AU	7.04 %	0.03 Rf	0.7 AU	185.4 AU	1.23 %
2	0.09 Rf	13.2 AU	0.15 Rf	26.0 AU	6.62 %	0.22 Rf	5.6 AU	1422.8 AU	9.47 %
3	0.26 Rf	0.1 AU	0.31 Rf	46.3 AU	11.81 %	0.32 Rf	30.8 AU	649.1 AU	4.32 %
4	0.32 Rf	31.8 AU	0.44 Rf	89.9 AU	22.91 %	0.45 Rf	89.5 AU	5375.6 AU	35.79 %
5	0.67 Rf	78.4 AU	0.73 Rf	100.7 AU	25.67 %	0.77 Rf	65.5 AU	5137.8 AU	34.21 %
6	0.88 Rf	70.0 AU	0.89 Rf	84.1 AU	21.45 %	0.95 Rf	29.7 AU	2038.4 AU	13.57 %
7	1.03 Rf	16.7 AU	1.03 Rf	17.6 AU	4.49 %	1.06 Rf	1.7 AU	208.8 AU	1.39 %

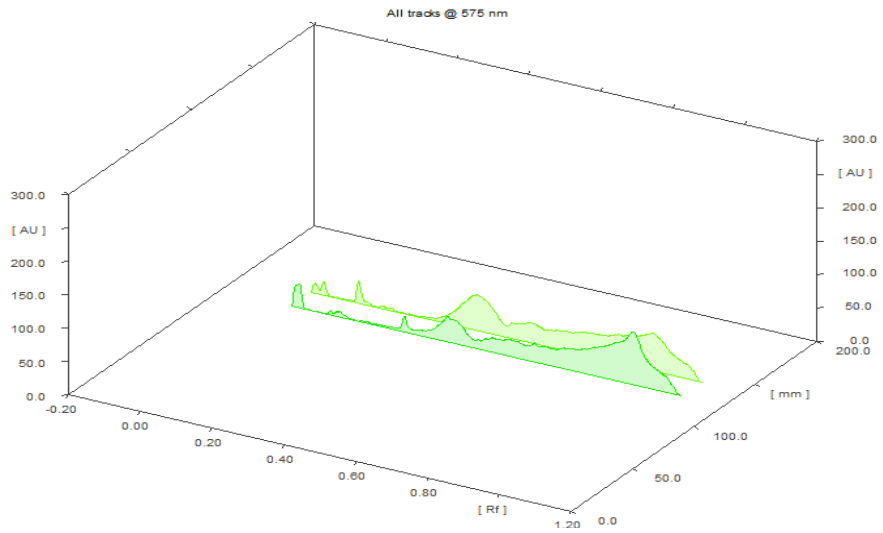


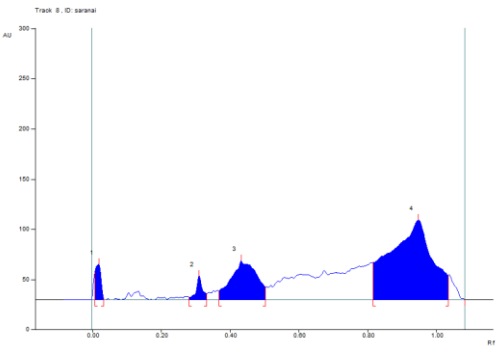
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	8.2 AU	0.01 Rf	39.2 AU	7.02 %	0.02 Rf	0.0 AU	268.9 AU	1.31 %
2	0.03 Rf	1.4 AU	0.04 Rf	21.1 AU	3.77 %	0.05 Rf	1.3 AU	120.0 AU	0.59 %
3	0.08 Rf	2.4 AU	0.12 Rf	56.7 AU	10.15 %	0.20 Rf	9.7 AU	1427.0 AU	6.97 %
4	0.20 Rf	10.0 AU	0.21 Rf	12.1 AU	2.16 %	0.23 Rf	1.7 AU	124.2 AU	0.61 %
5	0.29 Rf	3.9 AU	0.44 Rf	97.5 AU	17.44 %	0.45 Rf	93.3 AU	5801.9 AU	28.33 %
6	0.49 Rf	93.6 AU	0.51 Rf	97.4 AU	17.43 %	0.55 Rf	83.9 AU	3492.4 AU	17.05 %
7	0.69 Rf	85.1 AU	0.73 Rf	127.1 AU	22.75 %	0.78 Rf	66.0 AU	5521.8 AU	26.96 %
8	0.85 Rf	71.4 AU	0.86 Rf	75.8 AU	13.57 %	0.92 Rf	42.1 AU	2588.7 AU	12.64 %
9	0.97 Rf	30.0 AU	0.98 Rf	31.8 AU	5.69 %	1.06 Rf	1.3 AU	1134.4 AU	5.54 %

Figure 3. HPTLC fingerprint profile of10 µl and 5 µl of alcohol extract of Saaranai chooranam at 366 nm after derivatisation

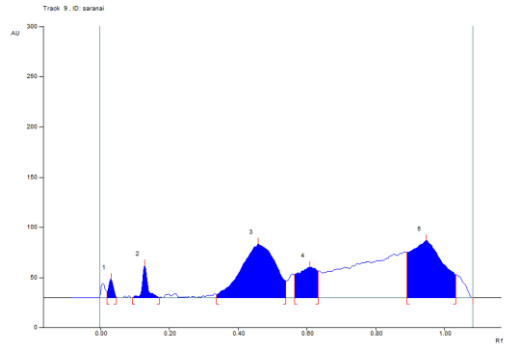
HPTLC finger printing analysis of alcoholic extract at 254 nm, the sample reveals the presence of 6 prominent peaks corresponds to the presence of 6 versatile phytocomponents present within it. Rf value of the peaks ranges from 0.00Rf – 1.03Rf. Further the peak 4 and 5 occupies the major percentage of area of 28.27% and 61.23% which denotes the abundant existence of such compound. HPTLC finger printing analysis of alcoholic extract at 366 nm, the sample reveals the presence of 9 prominent peaks corresponds to the presence of 9 versatile phyto components present within it. Rf value of the peaks ranges from 0.00Rf – 0.97Rf. Further the peak 5 and 7 occupies the major percentage of area of 28.83% and 26.96% which denotes the abundant existence of such compound.

575 nm





Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	27.5 AU	0.02 Rf	35.3 AU	19.95 %	0.03 Rf	1.0 AU	427.0 AU	4.58 %
2	0.28 Rf	2.7 AU	0.31 Rf	23.8 AU	13.43 %	0.33 Rf	6.0 AU	297.4 AU	3.19 %
3	0.37 Rf	8.9 AU	0.43 Rf	38.8 AU	21.93 %	0.50 Rf	12.8 AU	1997.5 AU	21.43 %
4	0.82 Rf	36.9 AU	0.95 Rf	79.1 AU	44.69 %	1.04 Rf	24.5 AU	6601.1 AU	70.80 %

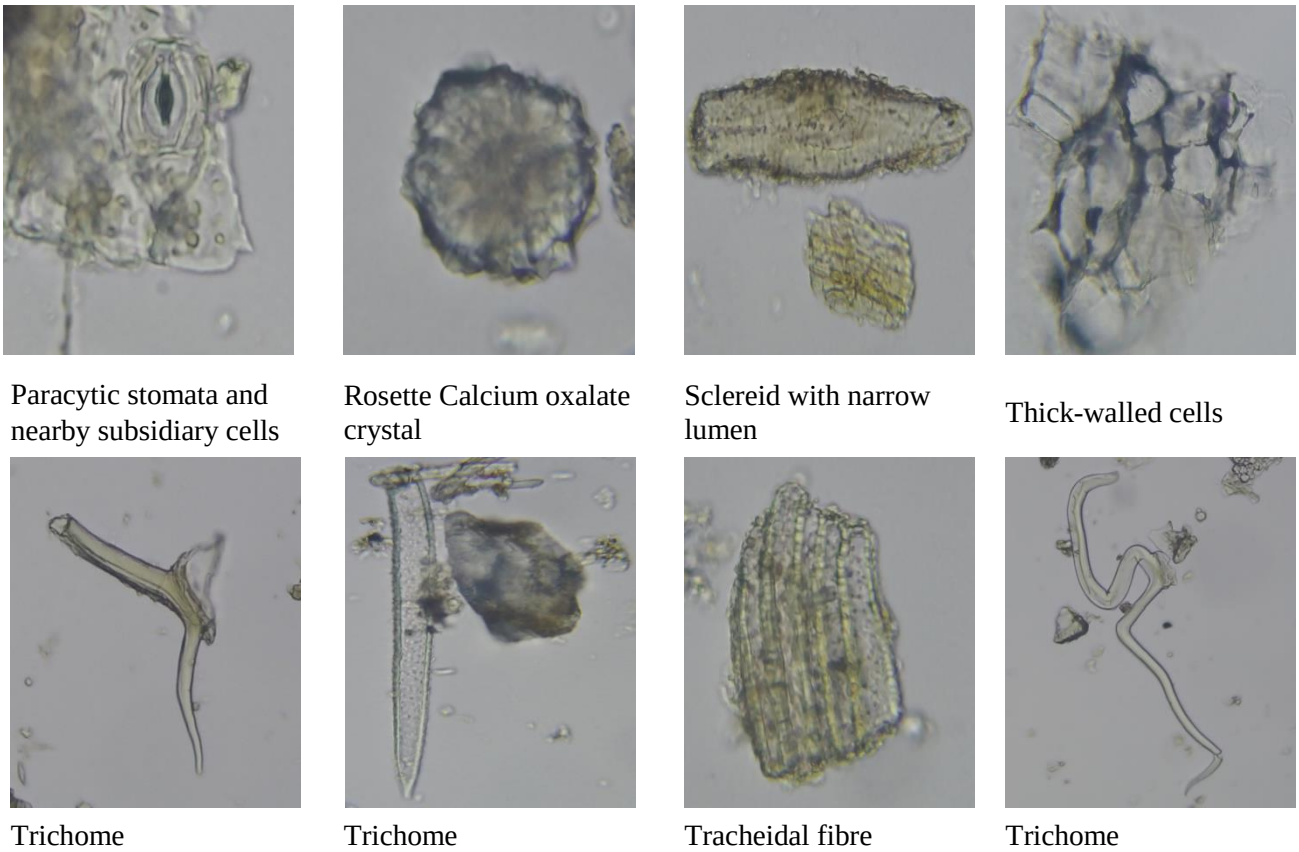


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	6.0 AU	0.03 Rf	18.6 AU	9.66 %	0.05 Rf	0.4 AU	175.0 AU	1.92 %
2	0.09 Rf	0.0 AU	0.13 Rf	32.4 AU	16.86 %	0.17 Rf	0.3 AU	348.3 AU	3.82 %
3	0.34 Rf	3.3 AU	0.46 Rf	53.7 AU	27.96 %	0.54 Rf	16.9 AU	3691.3 AU	40.52 %
4	0.56 Rf	23.3 AU	0.61 Rf	30.4 AU	15.82 %	0.63 Rf	26.5 AU	1148.4 AU	12.61 %
5	0.89 Rf	45.2 AU	0.95 Rf	57.1 AU	29.71 %	1.03 Rf	22.6 AU	3746.9 AU	41.13 %

Figure 4. HPTLC fingerprint profile of10 µl and 5 µl of alcohol extract of Saaranai chooranam at 575nm after derivatisation

HPTLC finger printing analysis of alcoholic extract at 575 nm, the sample reveals the presence of 5 prominent peaks corresponds to the presence of 5 versatile phyto components present within it. Rf value of the peaks ranges from 0.02Rf – 0.89Rf. Further the peak 3 and 5 occupies the major percentage of area of 40.52% and 41.13% which denotes the abundant existence of such compound.

4. Powder Microscopy



Paracytic stomata and nearby subsidiary cells

Rosette Calcium oxalate crystal

Sclereid with narrow lumen

Thick-walled cells

Trichome

Trichome

Tracheidal fibre

Trichome

Figure 5. Powder Microscopy

4.1. Powder microscopy interpretation

Numerous Tracheidal fibre, Vessel with bordered pits, Calcium oxalate crystal, Stone cell, Group of sclereids with brownish content, Paracytic stomata and surrounding subsidiary cells, Rosette Calcium oxalate crystal, Sclereid with narrow lumen, Thick walled cells, Tracheidal fibre and Trichome were present of Saaranai chooranam.

5. Conclusion

The above results revealed to helps in the correct identification and authentication may help to prevent adulteration, quality control of herbal products. The bio-active constituents like Proteins, Terpenoids, Alkaloids, Carbohydrates, Tannins are responsible for its therapeutic activity. Physicochemical evaluation of Saaranai Chooranam showed the values of Total ash, Sulphated ash, Ph of water extract, volatile oil, water soluble extractives, water soluble ash, swelling index and foaming index. High Performance Thin Layer Chromatography finger printing revealed the evidence of many phyto chemicals with different Rf values and densitometric scan of the plates showed numerous bands and peaks. The powder microscopy show specific microscopic characters using different staining reagents. The present study on phytochemical and physicochemical parameters, HPTLC analysis, Powder microscopy provides important information which can be used as a fingerprint for further studies in Saaranai Chooranam.

Declarations

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Competing Interests Statement

The authors have declared no competing interests.

Consent for Publication

The authors declare that they consented to the publication of this study.

Authors' Contribution

All authors took part in literature review, research, and manuscript writing equally.

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